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THE APPLICATION OF OPTICAL METHODS OF IDENTIFICATION TO ALKALOIDS AND OTHER ORGANIC COMPOUNDS.

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CONTENTS.

	Page.
Optical properties in the identification of synthetic crystalline substances	1
Methods for determination of optical constants:	
Apparatus	2
Observations	3
In ordinary light	3
Observations—Continued.	
In parallel polarized light	5
In convergent polarized light	6
Measuring the optical properties of an alkaloid	8
Summary	9

OPTICAL PROPERTIES IN THE IDENTIFICATION OF SYNTHETIC CRYSTALLINE SUBSTANCES.

Because crystals are best developed among minerals and were first studied in that connection, the sciences of crystallography and mineralogy have grown up in close association, and most of the printed information on crystallography is to be found in the literature dealing with mineralogy. Optical crystallography has been evolved largely for the identification of minerals, especially as they occur in rocks or as they are produced synthetically. To be sure, the crystallographic constants and partial optical data have been measured on a large number of artificial substances, both organic and inorganic, but little use has thus far been made of these observations for determinative purposes. Although microchemical methods of identification are used in a number of fields, the recorded data are largely qualitative. For instance, a given substance, when treated with an appropriate reagent, yields "radiating needles," "stout prisms," etc., numerical, quantitative observations being made only exceptionally.

"A careful study of much that has been written, and especially of the illustrations that have been made, of microcrystals in plants and drugs shows that erroneous conclusions may be easily drawn from the general appearance of crystalline precipitates or aggregates of crystals that are formed."[1] When it is considered how greatly crystal habit may be modified by the conditions of formation, the presence of impurities in the solutions, etc., the desirability of such quantitative data is apparent.[2]

A number of crystalline alkaloids, when obtained in a pure or nearly pure state, can be fairly well recognized by certain microchemical and color reactions. When mixed, however, and particularly when small amounts of one acccompany large amounts of another, it is difficult to establish the presence and identity of each. As there seemed every reason to hope that optical-crystallographic measurements would furnish a ready and certain means for the recognition of these substances, even when mixed in widely divergent proportions, a series of observations was made by the methods outlined in this bulletin. The results were satisfactory.[3]

METHODS FOR DETERMINATION OF OPTICAL CONSTANTS.

APPARATUS.

The instrument known as the petrographic microscope, because it was developed for use in the science of petrography, the study of rocks, is really an apparatus for the accurate measurement of crystallographic and optical constants of crystals in general. For instance, the system to which a crystal belongs, the axial ratios of the substance, the refractive indices, double refraction, and optical axial relations can be ascertained by its use. The determination of several such features on the same crystal will naturally be of enormously greater diagnostic value than the mere recording of more or less accidental shapes assumed by a crystalline substance under ill-defined conditions, which is all that is usually done. Moreover, it is not necessary that large or well-developed crystals be available. Most of the data can be readily measured on crystalline grains 0.025 millimeter ($\frac{1}{1000}$ inch) in diameter, and in favorable cases on even smaller ones, down to 0.01 millimeter ($\frac{1}{2500}$ inch), or, in other words, on what would ordinarily be classed as finely powdered materials.

While for the complete study and characterization of a crystallized substance a somewhat elaborate microscope outfit is necessary, comparatively few unusual accessories are needed to apply the methods herein described to the identification of alkaloids. The microscope must be equipped with nicol prisms which can be adjusted so that their vibration planes are exactly horizontal (right and left) and vertical (forward and backward); and an eyepiece with cross hairs in corresponding positions must then be used. It is a great convenience if one of these prisms can be rapidly inserted and withdrawn without changing the position of its vibration plane. A revolving stage, an Abbe condenser, and a substage iris diaphragm are necessary. One 4-millimeter ($\frac{1}{6}$-inch) and one 8-millimeter ($\frac{1}{3}$-inch) or 16-millimeter ($\frac{2}{3}$-inch) objective are adequate for every purpose. A slot should be cut in the microscope tube between the top of the objective and the bottom of the upper nicol prism for the insertion of a selenite plate yielding the "sensitive tint," red-violet of the first order. This plate, which may be purchased from any dealer in petrographic microscopes, is inserted so that its direction c lies at 45° from the vibration planes of the nicols. Finally, liquids of known refractive index are needed, directions for preparing which are given on page 4.

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[1] Kraemer, Henry. Applied and Economic Botany (Philadelphia, 1914), p. 768.

[2] Fry, W. H. Identification of Commercial Fertilizer Materials, 1914, U. S. Dept. Agri. Bul. 97. Wright, F. E. The Petrographic Microscope in Analysis, Jour. Amer. Chem. Soc., 1916, **38:** 1647–1658. Chamot, E. M. Chemical Microscopy, Jour. Ind. Eng. Chem., 1918, **10:** 60–66.

[3] To be published in the Jour. Amer. Chem. Soc.

OBSERVATIONS.

Samples are prepared by gently crushing the dry substance. A minute amount is immersed in a drop of a liquid in which it is insoluble, on a glass slide, and protected by a small cover glass. The observations are made as follows:

I. IN ORDINARY LIGHT.

A 16-millimeter ($\frac{2}{3}$-inch) objective is usually most satisfactory for the preliminary examination of a substance.

Color or hue.—If the substance is pleochroic, showing different colors in different crystallographic directions, the several colors and their directions are recorded.

Crystal habit.—This observation includes the outline of crystals, etc.

Angles.—Interfacial angles are measured by revolving the stage of the microscope until the trace of each face in turn lies parallel to one of the cross hairs, and reading the angular position of each. This process is repeated several times, and the results averaged.

Cleavage—Cleavage refers to the directions in which splitting along definite planes occurs.

Refractive indices.—These are inversely proportional to the rates at which light is transmitted in different crystallographic directions. They are determined by immersion in liquids of known indices, trying one after another until the boundaries in each direction successively disappear. The following special instructions are important: In crystals of the rhombic, monoclinic, and triclinic systems there are three indices, the smallest being called α, the intermediate β, and the largest γ. The directions in which these values hold are respectively X, Y, and Z. In substances of the tetragonal, trigonal, and hexagonal systems there are two indices, one called ω and the other ϵ. In the cubic system there is but one index, called n.

Determination of refractive indices by the immersion method.—A series of liquids of known indices should first be prepared, the composition of which depends on the nature of the substance to be examined. With the alkaloids studied, solutions of potassium-mercuric iodid in glycerol and water were found to be fairly satisfactory.

The stock solution is prepared as follows: Mix equal volumes of water and pure glycerol. In this dissolve 2.88 grams of potassium iodid and 3.38 grams of mercuric iodid per cubic centimeter of liquid; evaporate the solution on the water bath to the point of incipient crystallization; at 20° its index is approximately 1.71. Dilute this with glycerol (index 1.42) in the calculated proportions to make liquids with indices differing by a uniform amount, preferably 0.01. Measure the true indices of these liquids on a refractometer, or by other means, and correct the indices by the addition of glycerol or of stock solution, as necessary. The liquids are best kept in small dropping bottles, protected by glass caps.

A few crystal grains are immersed in a drop of one of the liquids on a slide, and the relative indices of grain and liquid determined thus: The light entering the microscope is diminished by means of the substage diaphragm, and a grain is brought into focus. Then, on raising the microscope tube slightly, a band of light passes into that substance—crystal or liquid—having the higher refractive index in that direction. The more divergent the indices of the two, the more brilliant the band; while when they are nearly matched, the band becomes faint, and, if white light is used, shows pale blue and orange margins. When the two have the same indices, no light moves in either direction. For accurate matching, monochromatic light, such as that from a sodium flame, should be used. White light passed through a ray filter is convenient. The writer uses a combination of Wratten E-red No. 23 and B–2 Extra Light No. 57–A filters, 3 inches square, in glass.

Crystals belonging to the cubic system have but one index, and their position is of no significance. Those of other systems, however, possess more than one, and the position in which they are observed must be taken into consideration. The grain to be studied should first be turned until it becomes dark or "extinguished" between crossed nicols. If the lower nicol prism, the polarizer, is inserted alone, two indices can be observed on any one grain, one when it is parallel, the other when it is perpendicular, to the direction of vibration of this nicol. If the elongation of the grain is positive, the highest index is shown when it is parallel, the lowest when perpendicular; if negative, the reverse relation holds. When the substance crystallizes in one of the less symmetrical systems, and has three different indices, grains lying in different crystallographic directions must be observed in order to measure all three.

II. IN PARALLEL POLARIZED LIGHT.

Parallel polarized light is produced by inserting the crossed nicol prisms.

Extinction (*the directions in which the crystal becomes dark*).—Extinction is said to be parallel when it occurs on bringing a long axis or side of a grain into parallelism with one of the cross hairs, and inclined when it occurs in some other position. When inclined, the angle between the axis or side and the cross hair is measured and recorded as the extinction angle. Several measurements are made, and the maximum value used. Crystals of the tetragonal, trigonal, hexagonal, and rhombic systems show normally parallel extinction, as do also monoclinic crystals elongated parallel to axis *b*. Crystals of the cubic system are dark in all positions, and are said to be isotropic; they show none of the phenomena hereafter listed.

Double refraction (*birefringence*).—This is the difference between the highest and lowest indices of refraction. It is described as weak, moderate, strong, etc., and is estimated by the order of the polarization colors shown when the grain is midway between two extinction positions.

Determination of the double refraction by order of polarization colors.—The polarization color obtained by placing any doubly-refracting crystal grain between crossed nicols is dependent on its thickness and the strength of its double refraction. Table 1 gives the colors with different thicknesses and double refractions.

TABLE 1.—*Colors observed with various thicknesses and double refractions.*

Color	Double refraction ×thickness.	Color.	Double refraction ×thickness.
First order:	*Mm.*	Third order:	*Mm.*
Black	0.00000	Light violet	0.00115
Gray	.00005	Light blue	.00120
White	.00020	Light green	.00135
Yellow	.00035	Light yellow	.00140
Orange	.00045	Light orange	.00145
Red	.00052	Light red	.00155
Red-violet[1]	.00055	Light red-violet	.00160
Second order:		Fourth order:	
Violet	.00057	Pale violet	.00170
Blue	.00065	Pale blue	.00180
Green	.00080	Pale green	.00190
Yellow	.00090	Pale yellow	.00200
Orange	.00095	Pale orange	.00210
Red	.00105	Pale red	.00230
Red-violet	.00110	Pale red-violet[2]	.00240

[1] Sensitive tint, shown by the selenite plate.

[2] Fifth order colors are extremely faint, and higher orders are white.

The selenite plate may be used to determine the order of the color, provided it is not too high. The plate is inserted, and the crystal is observed when parallel and again when perpendicular to its direction *c*. In one direction the color of the grain will be of lower order, in the other of higher order, than the selenite, which itself yields red-violet between the first and second orders. Even though the order, can not be determined on the crystal alone, the order of the combination of crystal and selenite can usually be told in one direction at least. As the selenite adds or subtracts just one order to or from the color produced by the crystal alone, the order of the crystal can thus be calculated. The thickness of the grain is best measured by focusing first on the top and then on the bottom, and noting how much the fine adjustment head has been turned.

Sign of elongation [1] (*positive or negative*).—The elongation is positive when the greater of any two indices lies in the direction of the longest axis or dimension of a given grain, and negative when the lesser of two lies in that direction. This may be ascertained by observing elongated grains immersed in a medium possessing the same mean refractive index, in light passing through but one nicol prism, of which the direction of vibration is known. This last property is most readily determined by observing a few needlelike crystals of a dark-colored variety of the mineral tourmaline. The darkest color is always shown when these lie with their long axis perpendicular to the vibration direction of the nicol prism. If the grain under observation shows the lowest refractive index when its long dimension is perpendicular to the vibration direction of the nicol, and the highest when parallel to this, its elongation is positive. In the reverse case it is negative. The sign may also be ascertained by the use of the selenite plate. Crystals showing as low double refraction as possible are turned so that their longer dimensions are parallel to direction *c* of the plate, which is placed so as to make an angle of 45° with the vibration planes of the nicol. If the polarization color is intensified, so that the crystal appears blue, or other color of a higher order than the red-violet due to the selenite plate, the elongation is positive; if the crystal appears yellow, white, or gray, that is, of lower order color than the red-violet field, the elongation is negative. Turning the crystal through 90° so that its long direction is perpendicular to axis *c* of the plate gives the opposite result, the color decreasing in order if the elongation is positive and increasing if it is negative.

III. IN CONVERGENT POLARIZED LIGHT.

Convergent polarized light is produced by raising the substage condenser as close to the slide as possible and using a high-power objective, preferably one of $\frac{1}{6}$ inch or 4 millimeters. After a grain is centered, the eyepiece is removed, or a Bertrand lens inserted, if the microscope is provided with one, and the stage, or nicol prisms, slowly revolved.

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[1] Strictly, "character of principal zone."

Class[1] may be *uniaxial*, possessing but one optic axis, or *biaxial*, possessing two. If biaxial, the angle between the two may be measured, for which purpose an apertometer or a micrometer eyepiece is needed. For the determinative work on alkaloids, however, it is necessary only to class the angle as small or large.

A uniaxial interference figure consists of a dark cross surrounded by rings which are colored if white light is used, or alternately dark and light in monochromatic light. It may appear in the center of the field if the crystal is lying on face *c*, the base, or it may lie more or less to the side. Even when the cross is not visible the dark arms will, on revolving the stage, move across the field parallel to each cross hair in turn, remaining perfectly straight.

A biaxial interference figure consists, in the most favorable case, of two dark spots, where the two optic axes emerge, surrounded by concentric oval or lemniscate curves, colored as in the uniaxial interference figure, and traversed by two dark bars which form a cross when the vibration directions of the crystal are parallel to those of the nicol prisms, and open out into hyperbolas when the stage is rotated 45°. The apparent angle, 2 E, is proportional to the distance separating these dark spots; the acute bisectrix lies midway between them. Two or three sheets of mica should be used for forming an idea of the appearance of the interference figures with different values of 2 E. Muscovite mica has 2 E from 60 to 70°; while phlogopite usually has 2 E much smaller, about 15°. In some positions only portions of the figure may be visible, but, in general, on revolving the stage the dark bars move across the field, neither parallel to the cross hairs nor remaining straight.

Optical sign[2] (*positive or negative*).—Uniaxial crystals are positive if ω is less than ϵ; negative if ω is greater than ϵ. Biaxial crystals are positive if Z is the acute bisectrix and negative if X occupies that position. This is determined in the same way as is the sign of elongation (page 6). That is, the selenite plate is inserted, and the change of color of the interference figure observed; when the figure shows, on opposite sides of the central cross, areas of blue, marking an increase in the order of the color in a direction parallel to *c* of the plate, the sign is positive; when the color is yellow in that direction, indicating a decrease, the sign is negative.

Dispersion.—Dispersion is the relative refraction of the red end of the spectrum as compared with the violet end, abbreviated to *r* and *v*, respectively. This can be observed in various ways, but, as it has in general little or no significance in the identification of alkaloids, it will not be further discussed here.

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[1] Strictly "ellipsoidity."

[2] Strictly "character."

MEASURING THE OPTICAL PROPERTIES OF AN ALKALOID.

Before measuring the optical properties of any substance, its purity must be established, for admixtures may modify some or all of its constants to a marked degree. The medium from which it is crystallized must also be taken into consideration, as some substances take up the liquids, in the form of "solvent-of-crystallization," and the crystallographic and optical characters may differ widely from one solvent to another. For instance, the alkaloid quinidine is recorded in the literature as crystallizing from acetone in the tetragonal system, being optically uniaxial, from methyl and other alcohols in the rhombic system, with axial ratios near 0.8:1:0.7 and with an optic axial angle (2 E) about 75°, and from benzene, also rhombic, but with the axial ratios nearer 0.7:1:1.1 and an optic axial angle of 85°. These differences are such that the crystals from these several solvents would be identified crystallographically or optically as belonging to different chemical substances by an observer not previously informed as to their character. Anyone desiring to gain familiarity with the manipulation of the microscope, and with the sort of effects upon which this method of identification of substances is based, may follow the directions outlined for the typical case described in the next paragraph.

Dissolve a 5-gram sample of cinchonine alkaloid in 100 cc of boiling 95 per cent alcohol. Filter the solution and allow it to cool, when the alkaloid is deposited on the walls of the vessel in small, brilliant crystals. Take out a few crystals, dry, crush, and mount them on a slide in potassium-mercuric iodid solution with a refractive index of about 1.61. Examined first with a low-power objective, in ordinary light, cut down by the substage diaphragm, the material shows plates and rods, many of them with hexagonal outlines. When the lower nicol is inserted, with its plane of vibration horizontal (right and left), all of the grains with their long dimensions horizontal stand out boldly, and light appears to pass into them as the tube is raised, indicating their refractive index lengthwise to be higher than that of the liquid. Many grains with their long dimensions vertical show faint orange and blue borders, indicating that the refractive index crosswise approximates that of the liquid, although, as this index is the mean between the two lower ones of the substance, it varies with the position in which each grain happens to lie. When both nicols are inserted and crossed, the diaphragm being now opened to obtain as bright an illumination as possible, the majority of the grains are white, or have hues of the fourth order, because of the extremely strong double refraction, with several marginal color bands, due to the wedge-shaped edges. A few grains show brilliant hues of lower orders, because of extreme thinness or because they are so turned that two refractive indices which are not very dissimilar are shown. When the stage is turned they, for the most part, become dark when their long dimensions are nearly or quite parallel with one of the cross hairs, although in some grains this extinction is not complete in any position. When a high-power objective is inserted and focused on a grain, the nicols remaining crossed, and the eyepiece is removed, nearly every grain shows a portion of a biaxial interference figure, colored rings, with one or two strongly curved dark bands crossing them as the stage is revolved. In some cases the points of emergence of both optic axes are in view simultaneously, and they are fairly close together, showing that the axial angle is small.

If it is desired to identify this substance in a drug mixture, the alkaloid contents may be extracted with chloroform, dried, taken up in benzene, and allowed to stand until the solvent is nearly evaporated. Single large drops of the concentrated solution are placed on several microscope slides, and immediately protected by cover glasses. Evaporation takes place around the edge of the covers, and there the alkaloids crystallize. When the benzene is completely volatilized, the immersion liquids may be introduced, and the appropriate observations made.

SUMMARY.

It seems reasonable to hope that optical-crystallographic measurements will furnish a ready and certain means for the recognition of a number of crystalline alkaloids, even when they are mixed in widely divergent proportions. The observations made on such substances with the apparatus and according to the methods described in this bulletin were most successful in establishing the identity of a number of these substances.

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